

## Patent Claims

1           1. A method of microbial production of amino acids of  
2 aspartate and/or glutamate families in which the pyruvate-  
3 carboxylase activity is increased by genetic modification of the  
4 enzyme and/or the pyruvate-carboxylase gene expression of the  
5 corresponding amino-acid-producing micro organism.

1           2. The method of claim 1, characterized in that, by  
2 mutation of the endogenous pyruvate-carboxylase gene an enzyme with  
3 higher pyruvate-carboxylase activity is produced.

A  
1           3. The method of claim 1 ~~or 2~~, characterized in that,  
2 the gene expression of the pyruvate-carboxylase is increased by  
3 increasing the gene copy number.

1           4. The method according to claim 3, characterized in  
2 that, to increase the gene copy number the pyruvate-carboxylase  
3 gene is incorporated in a gene construct.

1           5. The method according to claim 3, characterized in  
2 that, the gene is incorporated in a gene construct which contains  
3 regulatory gene sequences associated with the pyruvate-carboxylase  
4 gene.

1           6. The method according to claim 4 ~~or~~ 5, characterized  
2 in that, the corresponding amino-acid-producing microorganism is  
3 transformed with the gene-containing gene construct.

1           7. The method according to claim 6, characterized in  
2 that, a microorganism of the species *Corynebacterium* is transformed  
3 with the gene containing the gene construct.

1           8. The method according to claim 6 ~~or~~ 7, characterized  
2 in that, for the transformation a microorganism is used in which  
3 the enzyme participating in the synthesis of the corresponding  
4 amino acid is deregulated and/or wherein an enhanced export carrier  
5 activity is shown for the corresponding amino acid.

1           9. The method according to claim 6 ~~to 8~~, characterized  
2 in that, for the transformation a microorganism is used which has a  
3 higher proportion of the central metabolism metabolites of the  
4 corresponding amino acid participating in the synthesis.

1           10. The method according to claim 6 ~~to 9~~, characterized  
2 in that, for the transformation a microorganism is used in which  
3 biosynthesis paths competing with the corresponding amino acid  
4 biosynthesis paths runs with reduced activity.

1           11. The method according to ~~one of the preceding~~ claims, <sup>1</sup>  
2 characterized in that, the pyruvate-carboxylase gene is isolated  
3 from a microorganism strain of the variety *Corynebacterium*.

1           12. The method according to ~~one of the preceding~~ claims, <sup>1</sup>  
2 characterized in that, the gene expression is increased by  
3 reinforcement of the transcription signal.

1           13. The method according to ~~one of the preceding~~ claims, <sup>1</sup>  
2 characterized in that, the pyruvate-carboxylase gene has the tac-  
3 promot r ahead of the pyruvate-carboxylase gene.

1 14. The method according to claim 13, characterized in  
2 that, the tac-promoter is associated with regulatory sequences.

1 15. The method according to ~~one of the preceding claims~~<sup>1</sup>,  
2 characterized in that, the pyruvate-carboxylase gene is a gene with  
3 the amino acid sequence given under SEQ ID No. 2 and its allele  
4 variation coding nucleotide sequences.

1 16. The method according to claim 15, characterized in  
2 that, with the pyruvate-carboxylase gene a gene with the nucleotide  
3 sequence of nucleotide 165 to 3587 according to SEQ ID No. 1 or a  
4 substantially identically-effective DNA sequence is used.

1 17. The method according to ~~one of the preceding claims~~<sup>1</sup>,  
2 for the production of lysine, threonine, homoserine, glutamate  
3 and/or arginine.

1 18. A pyruvate-carboxylase gene coding for the amino  
2 acid sequence given under SEQ ID No. 2 and /or a nucleotide  
3 sequence coding for its allele variations.

1           19. The pyruvate-carboxylase gene according to claim 18  
2 with the nucleotide sequence of nucleotides 165 to 3587 according  
3 to SEQ ID No. 1 or a substantially identically-effective DNA  
4 sequence.

1           20. The pyruvate-carboxylase gene according to claim 18  
2 ~~or 19~~ with a preceding promoter of the nucleotide sequence from  
3 nucleotide 20 to 109 according to SEQ ID No. 1 or a substantially-  
4 identically-effective DNA sequence.

5           21. The pyruvate-carboxylate gene according to claim 18  
6 ~~or 19~~, with preceding tac-promoter.

7           22. The pyruvate-carboxylase gene according to claim 21  
8 with the regulatory sequence associated with the promoter.

1           23. The pyruvate-carboxylase gene according to ~~one of~~  
2 ~~claims 18 to 20~~ with these regulatory gene sequences associated  
3 therewith.

1           24. A gene structure containing a pyruvate-carboxylase  
2 gene according to ~~one of claims 18 to 23~~.

3           25. A vector containing a pyruvate-carboxylase gene  
4 ~~according to one of claims 18 to 23~~ or a gene structure according  
5 to claim <sup>18</sup>~~24~~.

1           26. Transformed cells containing in replicatable form a  
2 pyruvate-carboxylase gene ~~according to one of claims 18 to 23~~ or a  
3 gene structure according to claim <sup>18</sup>~~24~~.

1           27. Transformed cells ~~according to claim 26~~ containing a  
2 vector according to claim 25.

1           28. Transformed cells according to claim 26 ~~or 27~~,  
2 characterized in that, they belong to the variety *Corynebacterium*.

1           29. Transformed cells according to ~~one of claims 26 to~~  
2 ~~28~~, characterized in that, enzymes which participate in the  
3 synthesis of the corresponding amino acid and/or enzyme which  
4 participate in the export of the corresponding amino acid are  
5 deregulated.

6 30. Transformed cells according to ~~one of claims 26 to~~  
7 ~~29~~, characterized in that, they contain an increased proportion of  
8 the central metabolism metabolites participating in the synthesis  
9 of the corresponding amino acid.

1 31. Transformed cells according to ~~one of claims 26 to~~  
2 30, characterized in that, they contain a reduced proportion of the  
3 central metabolism metabolites which do not participate in the  
4 synthesis of the corresponding amino acid.

1 32. The use of a pyruvate-carboxylase gene for  
2 increasing the production of amino acids of the aspartate and/or  
3 glutamate families by microorganisms.

1 33. The use according to claim 32, characterized in  
2 that, a mutated pyruvate-carboxylase gene which codes for an enzyme  
3 with increase pyruvate-carboxylase activity is used.

1 34. The use according to claim 32 ~~or 33~~, characterized  
2 in that, the microorganism producing the corresponding amino acid

3 is transformed with a gene construct that contains a pyruvate-  
4 carboxylase gene.

1 35. The use according to claim 34, characterized in  
2 that, the gene construct additionally contains regulatory gene  
3 sequences.

1 36. The use according to ~~one of claims 32 or 35~~,  
2 characterized in that, a pyruvate-carboxylase gene from  
3 *Corynebacterium* is used.

1 37. The use according to ~~one of claims 32 or 36~~,  
2 characterized in that, *Corynebacterium* is used as the amino acid-  
3 producing microorganism.

Add cl